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(54) Title: METHOD FOR SOLUBILIZING KERATINACEOUS MATERIALS USING ALKALINE HYDROGEN PEROXIDE SOLUTION

(57) Abstract

Feathers, hair, wool and other materials which are composed substantially of keratin may be solubilized in alkaline solutions containing low concentrations of hydrogen peroxide. Solubilization is virtually complete at ambient temperatures and normal atmospheric pressures, yielding peptide products in excess of 75 % by weight of the keratin source. The peptides or amino acid mixtures obtained by hydrolysis of the peptides are rich in cysteine derivatives and are suitable for use as animal feed supplements. The solubilization process itself may be used therapeutically in human and veterinary medicine.

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METHOD FOR SOLUBILIZING KERATINACEOUS MATERIALS USING ALKALINE HYDROGEN PEROXIDE SOLUTION

Background of the Invention

The present invention relates to a chemical process for degrading keratinaceous materials.

Keratin is a fibrous protein which is characteristic of the skin or integument of animals and all the specialized derivatives of that integument: the hair, fur, hoofs, and nails of mammals, and the feathers and beaks of birds. These body tissues require high tensile strength to serve their purpose; moreover they must be resistant to solubilization, either aqueous, chemical or enzymatic. Keratin provides strength and structural stability to such tissues primarily because it has a high concentration of the amino acid cysteine. Cysteine molecules are able to combine with each other through stable disulfide bridges and in so doing link together different polypeptides or different parts of the protein molecule. Because keratin is cysteine-rich it is extensively cross-linked, tightly packed and dense. The effect is enhanced by the small size of its other major amino acids, glycine and serine.

Keratin is largely indigestible because its tight packing makes the peptide bonds of its constituent amino acids physically inaccessible to proteolytic enzymes of the digestive tract. For this reason it is not a useful food source; and keratinaceous materials, comprising a significant proportion of an animal's total protein, are often discarded or diverted to non-food uses by the meat processing industry.

The protein in these animal by-products could be made available by treatment of keratin to break its disulfide bonds and convert it to a soluble state in which peptide bonds are exposed. Chemical methods to solubilize keratin

include various oxidative or reductive treatments. Goodwin (U.S. Patent No. 3,970,614) used high concentrations (75-100%) of N,N-dimethylformamide and Matsuda (U.S. Patent No. 4,141,888) used urea or thiourea to solubilize feathers, fur, hair and hoofs. Kikkawa (U.S. Patent No. 4,135,942) describes the use of a reducing agent, sodium thioglycolate, or an oxidizing agent, performic acid, to attack the cysteine disulfide bonds of keratin. The disadvantages of these chemical methods are the cost of reagents and the necessity of separating reaction by-products from the solubilized keratin.

Kadri (U.S. Patent No. 4,172,073) describes the use of high pressure steam to solubilize keratinaceous materials. However, this non-chemical method requires expensive equipment and is inherently dangerous to workers.

It is accordingly an object of this invention to provide a method for solubilizing keratin and converting it into a digestible form which eliminates the need for expensive chemical or hazardous processes, and which does not leave a toxic residue in the product.

Solubilized keratin is a source of amino acids and is particularly rich in cysteine, which may be conveniently isolated from the other amino acids by conventional methods. Cysteine can then be converted into derivatives which have added value.

It is, therefore, a further object of the present invention to provide a method for producing cysteine, cysteic acid, and taurine from keratinaceous materials.

Certain skin disorders involve processes of hyperkeratinization which produce unsightly lesions. These lesions can be treated by solubilizing keratin in the skin. Keratinase enzymes have been used for this purpose, but they are expensive and, because of their

protein nature, have the capacity to raise an immune response in the patients.

It is therefore an object of the present invention to provide a method for topically reducing keratin in skin at low cost and with non-immunogenic agents.

Brief Summary of the Invention

We have discovered that keratinaceous materials may be solubilized by treating them with hydrogen peroxide in an alkaline aqueous solution under conditions of ordinary temperature and pressure. The treatment does not involve hazardous materials or leave toxic or unpalatable residues in foodstuff. Further, the principal reagent, hydrogen peroxide, can be produced cheaply from alcohol by an enzymatic process that we have developed that utilizes alcohol oxidase from Hansenula polymorpha.

In accordance with one aspect of the invention, there is provided a method for hydrolyzing keratinaceous materials, comprising contacting the keratinaceous material with an alkaline solution which contains hydrogen peroxide. In a preferred embodiment the alkaline solution is that of a hydroxide compound, such as NaOH, KOH or NH_4OH at a concentration sufficient to produce an initial pH of at least 9. In a particularly preferred embodiment, the alkaline solution has a pH of at least 11.

In another embodiment of the invention, the method further comprises the acid hydrolysis of peptides to amino acids. In a preferred embodiment, the acid hydrolysis of the peptides is carried out by means of a hydrochloric acid digestion. In yet another embodiment of the invention the method further comprises the isolation of specific amino acids from the acid hydrolysis product.

According to another aspect of the invention, the keratinaceous material is obtained from a vertebrate species, and in a preferred embodiment the material is obtained from avian or mammalian species. In a

particularly preferred embodiment, the keratinaceous material is feathers and down. In yet another embodiment of the invention the keratinaceous source material is wool or hair, and in yet other embodiments the keratinaceous material may be hides or skins of animals. Alternatively, the keratinaceous material may comprise fish skin or scales.

According to another aspect of the invention there is provided hydrolyzed protein which is produced by treating keratinaceous materials with alkaline hydrogen peroxide. The hydrolyzed protein produced comprises a mixture of polypeptides, oligopeptides and peptides which is usable as a feed supplement for amino acids. In a preferred embodiment there is provided a hydrolyzed protein which is derived from the feathers and down of birds. The invention further provides amino acids mixtures produced from the solubilization of keratinaceous material, followed by hydrolysis of protein and peptides to amino acids. The hydrolysis of the proteins and peptides may be carried out by treatment with acid, preferably hydrochloric acid, treatment with base, or digestion with proteolytic enzymes. The hydrolysis may also be carried out by continued treatment with an alkaline solution of hydrogen peroxide. In preferred embodiments, this amino acid mixture is derived from the protein of the feathers and down of birds or from fish meal.

According to another aspect of the invention there is also provided a method for solubilizing keratin by means of treatment with hot alkaline solution, preferably at a temperature of at least about 100°C.

According to another aspect of the invention there is also provided a method for hydrolyzing keratin in a zone in the skin of an animal which comprises contacting the zone of the skin with an alkaline solution of hydrogen peroxide and then allowing the solution to remain in

contact with the zone of the skin until the amount of keratin is substantially reduced or degraded.

In one embodiment the method is used to facilitate transdermal delivery of a therapeutic agent by hydrolyzing and solubilizing the keratin of skin to reduce the normal resistance of the zone of the skin to fluid penetration.

According to yet another embodiment the method is used in treating a dermatological disorder of hyperkeratinization by removing the keratin from a zone on skin by the method of alkaline hydrogen peroxide treatment described.

According to yet another aspect of the invention there is provided a medicament for topical use which comprises effective concentration of hydrogen peroxide together with an alkaline agent in an inert base.

According to yet another aspect of the invention, the alkaline hydrogen peroxide method may be used to solubilize keratinaceous material which has accumulated in drain lines and filters and which blocks the flow therethrough.

Detailed Description of the Invention

Alkaline solutions of hydrogen peroxide (H_2O_2) are capable of solubilizing keratinaceous materials so as to make the endogenous protein available as a food supplement, and as the source of other commercially valuable protein products. Keratinaceous materials which can be usefully treated comprise the feathers of wild and domestic fowl, including chickens, turkeys, ducks and geese, the hoofs, hides, horns, beaks, claws, scales, nails, skin, hair and wool and the membranes of egg shells. Much of this protein-rich material is now discarded as waste. Other salvageable keratinaceous material is found in discarded animal parts and eggs containing unborn chicks.

The present invention is described in terms of chicken feathers, an important waste by-product of the food industry, but it is understood the method is generally applicable to similar materials.

An alkaline solution containing as little as 1% H_2O_2 is effective in solubilizing common keratinaceous materials at ordinary room temperatures. The solution may be made alkaline by the addition of any basic substance, but preferably the base is a hydroxide of a monovalent cation, for example K^+, Na^+ or NH_4^+, whose neutralization products are relatively soluble and are harmless in food products.

The efficiency of the process is affected by pH, the presence of metal cations, and the ratio of solution to the keratin source. Solubilization of keratinaceous material is poor in hydrogen peroxide solutions where the pH is less than 9; however, when a solution of 1% H_2O_2 is brought to pH 10 by adding roughly 1% by weight of NaOH it can release over 80% of the weight of crude keratin chicken feathers as soluble protein (Example 3).

The presence of low concentrations of divalent metal ions in those solutions of Example 3 containing $FeSO_4$ and $MnCl_2$ apparently reduced the yield of protein solubilized from the keratin of feathers, presumably by decomposing H_2O_2 under the alkaline conditions. The weight ratio 5:1 for feathers:peroxide remained optimum for 2-fold differences in the weights of each. (Example 2)

Quantities of minced avian feathers treated for three days in 1% H_2O_2 solution containing 1% NaOH (pH 10) where the weight ratio between the feathers and H_2O_2 is 5:1, converted over 80% of this common keratinaceous material to soluble protein.

The present invention is superior to other keratin solubilization procedures in terms of costs, convenience and safety. Unlike the high pressure steam method used commercially to convert feathers to animal feed, the

alkaline peroxide method can be carried out at room temperature without using special equipment. It is superior to known chemical methods, such as hydrolysis with dimethylformamide (Goodwin U.S. Patent No. 3,970,614) which requires boiling under reflux with high concentrations of expensive solvent from which the protein product must then be extracted and separated by precipitation. It is also preferable to reducing hydrolysis which must be carried out in an atmosphere of inert gas. (Kadri U.S. Patent No. 4,172,073). The reagents used in alkaline peroxide solubilization, unlike those of alternate methods, are inherently safe when used at the low concentrations required. Hydrogen peroxide (which remains in the reaction mixture) decomposes to harmless oxygen and water. The NaOH remaining can be diluted out to reduce the pH of the reaction mix to neutrality or can be neutralized with HCl or acetic acid to harmless soluble salts.

Alkaline peroxide solubilization of keratinaceous material converts biological waste into available protein more efficiently than alternative processes.

Where solubilization of keratin is used to produce food protein, the measure of efficiency is not only the total protein recovery, but the conservation of inherent nutritional quality. The peroxide method conserves cystine in its oxidized state as cysteic acid. Autoclaving with NaOH destroys substantial amounts of serine, as well as arginine and threonine, two amino acids essential to animal nutrition. Although the alkaline peroxide method appears to destroy some methionine, the loss is compensated by the retention of cystine, because 80-90% of the dietary methionine requirement may be met by cystine (Rose, W. Nutr. Abstr. Rev. 27, 631 (1987).

Protein from solubilized keratin is important also as a source of peptides and amino acids. Once keratin has been converted to soluble protein by the action of

alkaline hydrogen peroxide on the disulfide bonds of its cysteine molecules, the protein may be further broken down to peptides and/or amino acids by known methods of proteolysis. The principal methods are acid or base hydrolysis and enzymatic digestion. Acid or base act non-selectively on peptide bonds to convert protein to its constituent amino acids. The proteolytic enzymes act selectively on a few of peptide bonds to convert proteins to peptides or oligopeptides, containing from a few to a substantial number of amino acids. The number and size of hydrolyzed peptides an enzyme produces is related to the enzyme's specificity. For example, trypsin has a specificity for peptide bonds involving lysine or arginine, and two less common acid cuids, so trypsin digestion produces a small number of relatively large peptides; by comparison pepsin hydrolyses peptide bonds involving six of the more common amino acids, and pepsin digestion produces a large number of relatively small peptide. Accordingly, solubilized keratin can be further hydrolyzed to any chosen extent by selecting the hydrolyzing agent appropriately.

It is also possible to carry out keratin solubilization and proteolytic hydrolysis in a single process step. Depending on the pH and the amount of peroxide available after the solubilization of keratin has begun, the reaction will proceed to protein hydrolysis. Depending on the amount of reagent excess provided at the start of the reaction and the time the reaction is allowed to proceed, the degree of hydrolysis of the original keratinaceous protein may be negligible, partial or complete.

These hydrolyzed protein products may be used for a variety of commercial purposes, non-nutritive as well as nutritive. As an example, of nutritive use the entire protein product from solubilized keratin may be added to a food product to improve its protein content. In preferred

embodiments, selected amino acids, usually the essential amino acids are isolated from protein hydrolysales derived from solubilized keratin and added to food products as supplements. Also, individual amino acids may be thus isolated for use as food supplements. The supplemented foods may be used in either animal or human nutrition.

The high concentration of cysteine in keratin makes its protein hydrolysates suitable for dietary supplements requiring this amino acid or related derivatives. Pet cats which have been fed some commercial cat foods may develop a degenerative disease of the myocardium leading ultimately to heart failure. This disorder can be reversed by feeding cats a diet supplemented in taurine. (Pion, P.D. et al. Myocardial Failure in Cats Associated with Low Plasma Taurine: A Reversible Cardiomyopathy, Science 237 p. 764 (1987)). Taurine is the decarboxylation product of cysteic acid, and conversion of the cysteine to taurine via decarboxylation may provide a food supplement for cats which supplies an abundant supply of taurine (Example 6).

The method of the present invention is a safe and effective means to solubilize skin keratin in situ in living animals, including humans, and consequently can be applied in various therapies. The keratin solubilization can be used as an adjunct therapy in ameliorating the discomforts of certain dermatological diseases. Non-inflammatory epidermal hyperplasia marked by a keratinaceous excrescence of skin or thickened stratum corneum without neovascularization are particularly appropriate for this type of treatment. Among these are the simple callous, senile keratosis, the quiescent parakeratosis and hyperkeratosis of psoriasis, ichthyosis congenital, acanthosis nigricans, chronic discoid lupus erythematosus, seborrheic keratosis, and keratosis follicularis.

The method can also be used to increase the transdermal drug delivery provided by drug-impregnated skin patches. Pre-treatment of the zone of skin to which the patch is to be applied will solubilize the keratin in the upper layers of the epidermis, allowing it to be dissolved away, and the dekeratinized skin will then offer less resistance to the uptake of the drug. In this application and the application to dermatological diseases described previously, the degree and rate of dekeratinization of the skin area can be controlled by factors such as the vehicle in which the H_2O_2 is applied, the concentration of H_2O_2 and the buffering pH.

Animal studies may be conducted to demonstrate the suitability of mild alkaline peroxide treatments for the solubilization of dermal keratins. Such modifications of keratin in situ may allow certain drugs to be delivered at greater rates transdermally and may reduce the keratin levels of the stratum corneum for the treatment of dermatological disorders listed earlier and/or the removal of wrinkles.

The preferred substrate for experimental dermal treatments is skin from the Yucatan hairless micro-pig (HMP) (Lavker, R.M., et al. 1988. J. Invest. Dermatol. 90:580) as described in Example 8. However, skin from other pigs or various rodents (especially "nude" varieties) may be used to examine the effects of alkaline peroxide treatments. These experimental systems are models for human studies.

Whole animal studies may be conducted on HMP or other organisms, using skin patches impregnated with various concentrations of hydrogen peroxide and sodium hydroxide. The alkaline peroxide formulations may be combined with suitable carriers, such as aqueous propylene glycol mixtures, in the patch and applied to the skin surface for varying lengths of time. The patch may comprise an occlusion chamber with gauze or other absorbent material

to hold differing amounts of the alkaline peroxide formulation. To assist in the penetration of the alkaline peroxide to the keratinaceous layers, the formulation may include detergents, solvents, soaps, and/or abrasives.

For increasing the skin permeability for drug delivery a preferred treatment time is 48 hours or less. A more preferred treatment time is less than two hours. Patches for the longer time points are placed on the animal earlier, so that all time points may be assayed together.

For removal of keratinaceous buildup in the skin longer-term treatments of the animals may be necessary, including chronic applications of the solubilization mixture. In this case a large area is subjected to mild alkaline peroxide in a stable cream formulation(s) or in many patches applied to the skin. At various time for the different formulations small (punch) biopsies are taken for histological examination of the keratinaceous layers.

Alkaline peroxide treatments may also remove keratinaceous layers of the stratum corneum to reduce wrinkles. In this manner alkaline peroxide may substitute in part for drugs, such as retinoid acid (Retin-A; for a mini-review see Roberts, L. 1988. Science 239:564) and other vitamin A derivatives which interfere with the synthesis of skin keratins and thereby reduce the level of wrinkles. Alkaline peroxide treatment may also reduce cross-linking of non-keratinaceous substances, such as collagen or lipoproteins, to increase the flexibility of the skin.

The ability of alkaline peroxide treatment to solubilize the keratin in the hair shaft can be exploited in various applications. First, it can be adapted for cosmetic use in the straightening or permanent waving of hair. Both of these processes require a preliminary step in which the hair keratin conformation is relaxed by breaking the disulfide cystine bonds. After the hair is

mechanically re-conformed, the cystine bonds are rejoined, so that the new conformation is fixed.

The method can also be used cosmetically in depilation or removal of hair from skin by allowing the process to proceed until the hair shaft is destroyed. More vigorous depilation conditions, optionally accompanied by mechanical scrubbing or abrasion can adapt the method to the removal of hair from animal hides.

The solubilization method further has obvious uses in destroying hair or other keratinaceous material that forms an obstruction in any mechanical orifice, whether in bulk as a plug in plumbing drains, or as fine particles of hair which reduce the efficiency of combing, shearing or shaving instruments.

EXAMPLE 1

Solubilizing Chicken and Turkey Feathers

Unwashed chicken and turkey feathers were cut with scissors to lengths of approximately one-half inch to facilitate an accurate weighing of the feathers. A quantity of 1.0 gram of cut feathers was placed into each of five 250 ml Erlenmeyer flasks, labeled as indicated.

50 ml of the appropriate solution was then added to each flask. Flasks 1, 2, and 3 were placed on gyrator shakers (140 RPM) at ambient room temperature for three days. Flasks 4 and 5 were autoclaved at 15 psi, 120° C. for 20 minutes. Substantial solubilization of the feathers was visually noticeable in flask 3 after the first day of incubation and in flask 4 immediately after treatment.

The contents of flasks 1, 2, 3, and 4 were filtered by a Buchner funnel through Whatman #1 paper, and the collected fluids were assayed for protein content by the method of Lowry, et al., 1951, J. Biol. Chem., 193:265. with bovine serum albumin (BSA) as a standard. The liquid

content of flask 5 was measured directly for protein levels using the same assay.

Alkaline hydrogen peroxide treatment of the feathers yielded the highest amount of assayable protein; autoclaved NaOH treatment gave protein values that were 23% lower. The other regimens were far inferior.

TREATMENT	SOLUBLE PROTEIN
1. Water Only	5.0 mg
2. 1%NaOH	165.6 mg
3. 1%NaOH + 1%H_2O_2	818.0 mg
4. 1%NaOH - Autoclaved	629.8 mg
5. Water only - Autoclaved	12.3 mg

SDS Polyacrylamide gel electrophoresis of the liquid in flasks 3 and 4 showed the products therein to be Coomassie Blue-staining peptides of 11,000 Daltons or smaller (Data not shown). A substantial fraction of each sample may be oligopeptides and/or free amino acids. Amino acid analysis of the solubilized proteins was carried out (see Example 4).

EXAMPLE 2

Optimization of Substrate-to-Peroxide Ratio

Various quantities of cut feathers were weighed and placed into flasks containing 10 ml of 1% NaOH and either 0.1 or 0.2 g of H_2O_2. After three days of rotary shaking at ambient temperature, the flasks were removed and the liquid portions of the samples assayed for total protein as in Example 1. The protein yield was then calculated.

FLASK NO.	FEATHERS	H_2O_2	PROTEIN RELEASED	YIELD (%)
1	0.2 g	0.1 g	150.4 mg	75.3
2	0.5	0.1	397.1	79.4
3	1.0	0.1	637.6	63.8
4	1.5	0.1	456.2	30.4
5	2.0	0.1	346.6	17.3
6	3.0	0.1	349.0	11.6
7	1.0	0.2	821.0	82.1
8	2.0	0.2	584.8	29.2
9	3.0	0.2	356.2	11.9

Under the conditions given, an optimum weight ratio of 5:1 for feathers:peroxide was observed. At this ratio approximately 80% of the substrate is converted to water-soluble protein (as detected in a Lowry assay).

EXAMPLE 3

Effect of pH and Metal Ions on Solubilization of Keratin in Feathers in 1% Hydrogen Peroxide Solution

A quantity of 400 mg of avian feather clippings were placed in each of several flasks containing a volume of 20 ml of 1% H_2O_2. The solutions in these sets of flasks were adjusted to acidic, neutral, and alkaline pH respectively, and Fe^{+2} and Mn^{+2} compounds added to sets of solutions at the various pH levels as indicated in the table. The feathers were treated in these solutions for 3 days, at which time the soluble protein concentration was determined.

Protein Released (mg)

Sample	pH: 4.2	7.0	10.5
No Metal	4.7	4.7	307.5
0.5 mM $FeSO_4$	5.6	4.1	295.1
2.0 mM $MnCl_2$	4.8	3.6	122.3

Following the initial period of treatment, the sensitivity of the process to pH was explored by acidifying the pH 4.2 samples to pH 2.5, and alkalinizing the pH 7.0 samples to pH 9.0. After three additional days of treatment, there was no increase in solubilization of the feathers as determined by visual observation.

EXAMPLE 4

Amino Acid Analysis of Alkaline Peroxide-Treated Feathers

The solubilized proteins from flasks 3 and 4 in Example 1 were acid-hydrolyzed with hydrochloride vapors in sealed, air-evacuated glass tubes at 110° C. for 24

hours. Amino acid determinations for this hydrolysate were carried out by the PTC method on a Hewlett Packard 1091A HPLC. Absorbances were read at 269 nm, and the data were analyzed (integrated) by a Nelson Data System computer program. Sample peak areas generated by HPLC were converted to amino acid concentrations using concentration/area ratios of known standards.

An amino acid composition for alkaline-hydrogen peroxide treated samples and for autoclaved alkaline treated feathers is given in the table.

AMINO ACID COMPOSITION OF PROTEIN SOLUBILIZED FROM AVIAN FEATHERS

	MOLAR %		WEIGHT %	
AMINO ACIDS	H_2O_2+NaOH	Hot NaOH	H202+NaOH	Hot NaOH
Alanine	8.980	12.977	7.034	10.335
Arginine	4.436	0.281	6.793	0.438
Asparatate	1.482	1.726	1.734	2.053
Cysteine	N.D.	N.D.	N.D.	N.D.
Glutamate	3.076	3.884	3.977	5.107
Glycine	16,520	19.988	10.906	13.418
Histidine	1.191	0.801	1.625	1.111
Isoleucine	3.036	3.649	3.501	4.279
Leucine	6.050	6.913	6.978	8.108
Lysine	0.608	0.896	0.781	1.171
Methionine	0.041	0.457	0.053	0.610
Phenylalanine	3.662	4.260	5.318	6.291
Proline	20.436	22.530	20.676	23.180
Serine	13.849	6.618	12.794	6.217
Threonine	2.071	0.191	2.169	0.203
Tryptophan	N.D.	N.D.	N.D.	N.D.
Tyrosine	3.711	6.371	5.912	10.318
Valine	5.326	6.116	5.482	6.402
CMCys	0.073	0.000	0.115	0.000
NH3	2.993	2.130	0.448	0.324
CS-SC	0.076	0.179	0.160	0.385
CYS03	2.383	0.032	3.544	0.048
Total	100.000	100.000	100.000	100.000

EXAMPLE 5

Solubilizing Wool and Other Hair

Undyed wool yarn was cut into lengths of approximately one inch and weighed into 1.0 gram portions. Each gram was placed into a separate 250-ml Erlenmeyer flask and subjected to a treatment regimen as in Example One, except that the room temperature reactions were terminated after two days by placing the flasks into a 4°C. coldbox.

The unfiltered liquid content of each flask was measured for soluble protein by the method of Lowry with BSA as standards. The following table gives the total amount of protein that was solubilized from one gram of wool by each treatment.

Water Only	1.4 mg
1% NaOH	413.2 mg
1% NaOH + 1% H_2O_2	980.4 mg
1% NaOH - Autoclaved	840.5 mg
Water Only - Autoclaved	13.4 mg

EXAMPLE 6

H_2O_2-Treated Feathers as a Cat Food Supplement

ESSENTIAL AMINO ACIDS	Observed g/kg	FEATHER AA RATIO	RECOMMENDED AA RATIO	CATFOOD[1] REQs (g/kg)
Arginine	63.97	14.06	15.50	10.0
Histidine	3.04	0.67	4.65	3.0
Isoleucine	50.03	11.00	7.75	5.0
Leucine	88.48	19.45	18.60	12.0
Lysine	6.96	1.53	12.40	8.0
Methionine	2.13	0.47	4.65	3.0
Phenylalanine	61.43	13.51	13.18	8.5
Threonine	37.84	8.32	10.85	7.0
Tryptophan		?	2.33	1.5
Valine	74.12	16.30	9.30	6.0
$CySO_3$/Taurine	66.83*	14.69	0.78**	0.5
Total	454.83			64.5

1 (National Research Council)

* Observed Cysteic Acid Levels

** Recommended amt. of Taurine (Nat'l Res. council)

EXAMPLE 7

Quantitation of Cysteine and Cysteate in Solubilized Feathers

Cysteine accounts for approximately 8% of feather amino acids by weight. However, cysteine is not directly quantifiable by the PTC methods. To stabilize cysteine for amino acid analysis the sample may be treated with performic acid to oxidize cysteine to cysteic acid, which may then be measured by the PTC method. The total amount of sulfur-containing amino acids in the protein product is an important parameter for use of the solubilized keratin as a food supplement. In addition, cysteic acid is an intermediate in the formation of taurine from cysteine and may be a dietary substitute for taurine, since taurine may be generated from cysteic acid by decarboxylation.

One gram of feathers was solubilized in 50 ml of 1% NaOH-1% H_2O_2, as described in Example 1. The pH of the solution was neutralized with HCl (from pH 12.8 to 6.8); and the mixture was taken to a total volume of 20 ml with distilled water. The protein concentration was estimated at 8 mg/ml, based upon earlier observations. 100 ug of this neutralized product was treated with performic acid (as described below) to demonstrate that virtually all of the cysteine had been converted to cysteic acid as a result of the alkaline peroxide treatment.

Performic acid was generated by mixing 450 ul of 30% H_2O_2 with 50 ul of 88% formic acid at -10°C. and incubating this reaction at -10°C for 2 hr in a saltwater-ice bath. 12.5 ul of the neutralized product (100 ug of protein) was added to the 500 ul of performate and held at -10°C. As a control, 12.5 ul of product was incubated with 500 ul of distilled water at the same temperature. After two hours, 2 ml of distilled water was added to each sample and the samples were frozen at -20°C. Both samples were lyophilized and analyzed for amino acid composition as previously described.

The following tables summarize the amino acid composition of 1) performate-treated product and 2) untreated product. In each case the cysteic acid levels are approximately 7% of the solubilized keratin. This observation indicates that nearly all of the cysteine had been converted to cysteic acid by the alkaline peroxide treatment. However, the secondary performate regimen slightly to increase the amount of cysteic acid in the solubilized keratin. Performate treatment appears to stabilize certain amino acids. The total amount of sulfur-containing amino acids was found to be 8.9% by weight in this study of solubilized feathers.

AMINO ACID COMPOSITION OF SOLUBILIZED FEATHERS

CONTROL: NOT PERFORMATE-TREATED

AMINO ACIDS	picoMoles	Molar%	picoGrams	Weight%
Alanine	914.21	6.822	81456	5.042
Arginine	593.24	4.427	102244	6.397
Aspartate	400.78	2.991	53344	3.302
Cysteine	N.D.	N.D.	N.D.	N.D.
Glutamate	1284.70	9.586	188980	11.698
Glycine	1799.98	13.431	135178	8.368
Histidine	31.59	0.236	4903	0.304
Isoleucine	616.02	4.597	80821	5.003
Leucine	1089.48	8.129	142940	8.848
Lysine	76.93	0.574	11248	0.696
Methionine	23.11	0.172	3448	0.213
Phenylalanine	600.67	4.482	99232	6.143
Proline	1596.15	11.910	183717	11.373
Serine	2005.97	14.968	210828	13.051
Threonine	513.24	3.830	61126	3.784
Tryptophan	N.D.	N.D.	N.D.	N.D.
Tyrosine	64.32	0.480	11656	0.722
Valine	1022.49	7.630	119734	7.412
CMCys	0.00	0.000	0	0.000
NH3	71.17	0.531	1212	0.075
Cystine	59.58	0.445	14317	0.886
Cysteic Acid	638.02	4.761	107953	6.683
TOTAL	13401.74	100.000	1615445	100.000

AMINO ACID COMPOSITION OF SOLUBILIZED FEATHERS
PERFORMATE-TREATED

AMINO ACIDS	picoMoles	Molar%	picoGrams	Weight%
Alanine	673.86	7.303	60041	5.501
Arginine	367.17	3.979	63961	5.860
Aspartate	393.48	4.264	52372	4.798
Cysteine	N.D.	N.D.	N.D.	N.D.
Glutamate	879.89	9.536	129432	11.859
Glycine	1461.22	15.836	109738	10.054
Histidine	26.58	0.288	4125	0.378
Isoleucine	401.44	4.351	52669	4.826
Leucine	713.61	7.734	93626	8.578
Lysine	60.20	0.653	8802	0.806
Methionine	19.59	0.212	2922	0.268
Phenylalanine	242.75	2.2631	40102	3.674
Proline	958.07	10.383	110274	10.103
Serine	1388.06	15.044	145885	13.366
Threonine	361.17	3.914	43015	3.941
Tryptophan	N.D.	N.D.	N.D.	N.D.
Tyrosine	0.00	0.000	0	0.000
Valine	676.32	7.330	79197	7.256
CMCys	0.00	0.000	0	0.000
NH3	83.39	0.904	1420	0.130
Cystine	82.39	0.893	19798	1.814
Cysteic Acid	437.72	4.744	74063	6.786
TOTAL	9226.99	100.000	1091452	100.000

EXAMPLE 8

Solubilization of Skin Keratins in Situ

Epidermis of the Yucatan hairless micro-pig (HMP) is treated with 0 to 50% hydrogen peroxide in 0 to 5% sodium hydroxide solution for time points up to 48 hours. The treated skins are tested for permeability at 35-37°C. in Franz diffusion chambers (Franz, T.J. 1978. Curr. Prob. Dermatol. 7:58-68) or other two-chambered apparati with isotope-labelled drugs, such as ^{14}C-diazepam or ^{3}H-hydrocortisone, or other compounds as diagnostic markers of diffusion across the membranes. Skin biopsies (about 4mm) are taken, and sections are made to examine histological changes in the stratum corneum. Alternatively, treated animals are sacrificed; and the skins are tested for permeability in the diffusion chambers noted above.

As described above, HMP skins were treated with 15% hydrogen peroxide in 4% sodium hydroxide solution for 30 minutes. Water was used as a control on other skins. Alkaline peroxide treated skin showed 4 times the permeability of hydrocortisone compared to the control skin in a time course of 24 hours.

WHAT IS CLAIMED:

1. A method for hydrolyzing keratinaceous materials, comprising:

contacting said keratinaceous material with an alkaline solution containing an effective keratin-hydrolyzing amount of hydrogen peroxide; and

permitting said alkaline solution to at least partially solubilize said keratinaceous material.

2. The method of Claim 1, wherein said alkaline solution contains a hydroxide compound.

3. The method of Claim 1, wherein said solution is made alkaline by the addition of a member of the group consisting of NaOH, KOH or NH_4OH.

4. The method of Claim 1, wherein said alkaline solution comprises a mixture of hydroxide compounds.

5. The method of Claim 1, wherein said solution has a pH of at least about 9.

6 The method of Claim 1, wherein said solution has a pH of at least about 11.

7. The method of Claim 1, wherein the amount of hydrogen peroxide in said solution is at least 0.1% by weight.

8. The method of Claim 1, further comprising the hydrolysis of the protein and peptides of said solubilized keratinaceous material to amino acids.

9. A method according to Claim 8, comprising acid hydrolysis of said proteins and peptides.

10. A method according to Claim 9, comprising hydrochloric acid hydrolysis.

11. A method according to Claim 8, comprising alkaline hydrolysis.

12. A method according to Claim 8, comprising hydrolysis using proteolytic enzymes.

13. A method according to Claim 8, comprising hydrolysis of said proteins and peptides by continued treatment with alkaline hydrogen peroxide solution.

14. A method according to Claim 8, further comprising the step of isolating a specific amino acid from said hydrolyzed protein and peptides.

15. A method according to Claim 1, wherein said keratinaceous material is from a vertebrate animal species.

16. A method according to Claim 15 wherein said keratinaceous material is from an avian species.

17. A method according to Claim 15, wherein said keratinaceous material comprises feathers, down, or feather meal.

18. A method according to Claim 15, wherein said keratinaceous material comprises wool or hair.

19. A method according to Claim 15, wherein said keratinaceous material comprises hides or skins.

20. A method according to Claim 15, wherein said keratinaceous material comprises fish scales or fishmeal.

21. A method of hydrolyzing protein to amino acids by contacting said protein with an alkaline solution containing at least 0.1% hydrogen peroxide.

22. A composition of matter produced by the method of Claim 1, comprising a mixture of polypeptides, oligopeptides, peptides, amino acids, and derivatives thereof.

23. The composition of Claim 22, wherein said composition is derived from feathers and down.

24. The composition of Claim 22, wherein said composition is derived from wool or hair.

25. The composition of Claim 21, wherein said composition is derived from fish scales or fish meal.

26. An amino acid mixture produced by the method of Claim 8.

27. An amino acid mixture of Claim 26, wherein said amino acid mixture is derived from the proteins of feathers and down.

28. An amino acid mixture of Claim 26, wherein said amino acid mixture is derived from the protein of wool or hair.

29. An amino acid mixture of Claim 26, wherein said amino acid mixture is derived from fish scales or fish meal.

30. An edible substance comprising at least 0.1% by weight of the mixture of Claim 21 or 24 as a dietary supplement.

31. The edible substance of Claim 30 as a human dietary product.

32. The edible substance of Claim 30 as an animal feed product.

33. A method for hydrolyzing keratin in a zone of the skin of an animal including a human, comprising:

contacting a zone of skin with an alkaline solution containing an effective keratin-hydrolyzing amount of hydrogen peroxide; and

allowing said solution to remain in contact with said zone until a predetermined amount of the keratin therein is solubilized.

34. A method according to Claim 33 for facilitating transdermal delivery of a therapeutic agent to an animal, including a human, wherein the solubilization of the keratin reduces the normal resistance of said zone of skin to fluid penetration.

35. A method according to Claim 33 for treating a dermatological disorder involving hyperkeratinization.

36. The method of Claim 33, wherein said animal is a human and said zone of skin is wrinkled or creased, and the solubilization of keratin therein is effective in reducing the degree of wrinkling or creasing.

37. A medicament for topical use comprising hydrogen peroxide together with an alkaline agent in a pharmacologically acceptable vehicle.

38. A method for hydrolyzing keratinaceous material, comprising:

contacting said keratinaceous material with a solution of an effective amount of a compound selected from the group consisting of NaOH, KOH or NH_4OH, said solution being heated to a temperature of at least about 80°C;

permitting said solution to at least partially solubilize said keratinaceous material while maintaining said temperature.

39. The method of Claim 38, wherein said solution is heated to at least 100°C.

40. The method of Claim 1, wherein said keratinaceous material is within a conduit for liquid waste.

41. The method of Claim 1, wherein said keratinaceous material is on the surface of a filter for liquid substances.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/03100

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4] C07C 99/02;C12P 21/06; A61K 33/40; A23J 1/02; B05B 9/00
530/343,357;562/445, 516, 559-563, 570, 573, 595
435/69,106,107,108,109,110,113,114,115,116,424/62,71,130;8/161;426/55,657,442;134/2,22.13

II FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.CL.	530/343,357;562/516;435/69;424/62,71,130;8/161;426/55,657,442 134/22.13,2;514/947

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	US,A, 2,158,499 (Grassmann et al.) 16 May 1939. See column 1, lines 6-19, and Example 13.	1-8,12-32, 38,39
X	US,A. 2,719,813 (Haefele) 4 October 1955 See Example VII.	1-5,7,15, 18,22,24, 33,37
X	US,A, 3,464,825 (Anker) 2 September 1969 See Example I.	1-8,11,13, 15-31,38, 39
X	US,A, 4,438,102 (Ganci) 20 March 1984 See Example 1	37
X	US,A,4,705,682 (Moeller et al.) 10 November 1987 See Example 1	38-39
X	US,A,4,664,836 (Taylor et al.) 12 May 1987 See column 2, lines 25-44	1-8,11,13, 15,18,21, 22,24,26, 28,38-41

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
19 September 1989	26 DEC 1989
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Carolyn S. Elmore Carolyn S. Elmore

Form PCT/ISA/210 (second sheet) (Rev.11-87)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X	US,A, 4,088,596 (Arai et al) 9 May 1978 See column 2, lines 19-38	1-6,11,13 15,18,21, 22,24,26, 28,38-41
Y	JP,A,61-68,426 (Fuji Oil KK) April 8,1986 See abstract	14

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . , because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ..... . , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.